Received: 29 August 2023 | Revised: 25 October 2023 | Accepted: 31 October 2023

DOI: 10.1002/psp4.13082

TUTORIAL

A tutorial for model-based evaluation and translation of cardiovascular safety in preclinical trials

Victoria Kulesh[1,2] | **Igor Vasyutin**[1] | **Alina Volkova**[1,3] | **Kirill Peskov**[1,2,3] | **Holly Kimko**[4] | **Victor Sokolov**[1,3] | **Ravindra Alluri**[5]

[1]Modeling & Simulation Decisions FZ-LLC, Dubai, United Arab Emirates

[2]Research Center of Model-Informed Drug Development, Sechenov First Moscow State Medical University, Moscow, Russia

[3]Sirius University of Science and Technology, Sirius, Russia

[4]CPQP, CPSS, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, Maryland, USA

[5]CPQP, CPSS, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK

Correspondence
Victor Sokolov, M&S Decisions FZ LLC, Dubai, United Arab Emirates.
Email: victor.sokolov@msdecisions.tech

Ravindra Alluri, Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca, Cambridge, UK.
Email: ravindra.alluri@astrazeneca.com

Abstract

Assessment of drug-induced effects on the cardiovascular (CV) system remains a critical component of the drug discovery process enabling refinement of the therapeutic index. Predicting potential drug-related unintended CV effects in the preclinical stage is necessary for first-in-human dose selection and preclusion of adverse CV effects in the clinical stage. According to the current guidelines for small molecules, nonclinical CV safety assessment conducted via telemetry analyses should be included in the safety pharmacology core battery studies. However, the manual for quantitative evaluation of the CV safety signals in animals is available only for electrocardiogram parameters (i.e., QT interval assessment), not for hemodynamic parameters (i.e., heart rate, blood pressure, etc.). Various model-based approaches, including empirical pharmacokinetic-toxicodynamic analyses and systems pharmacology modeling could be used in the framework of telemetry data evaluation. In this tutorial, we provide a comprehensive workflow for the analysis of nonclinical CV safety on hemodynamic parameters with a sequential approach, highlight the challenges associated with the data, and propose respective solutions, complemented with a reproducible example. The work is aimed at helping researchers conduct model-based analyses of the CV safety in animals with subsequent translation of the effect to humans seamlessly and efficiently.

INTRODUCTION

Nonclinical safety pharmacology studies are an essential part of the drug development workflow.[1] The S7A guideline of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use emphasizes the importance of identification and evaluation of undesirable pharmacodynamic (PD) properties of new substances on the cardiovascular (CV) system prior to first administration in humans. Of note, CV-related adverse drug reactions, including treatment-mediated cardiac rhythm disturbances, changes in arterial blood pressure, and thromboembolic complications, stand out as one of the most common causes of market withdrawal.[1,2] As the projects approach toward candidate selection, assessments are made using in vitro assays and

Victoria Kulesh and Igor Vasyutin contributed equally to the work.

in vivo studies, as to whether CV safety risks are acceptable in the context of species-species effects and predicted therapeutic margins.[3] Nonclinical CV safety assessment includes telemetry studies in conscious animals where continuous data of electrocardiogram (ECG; e.g., QT interval) and hemodynamic (e.g., heart rate, blood pressure, and contractility of the ventricles) parameters are collected, and warrants model-based analyses to predict potential unintended CV effects.[1,4] Currently used modeling and simulation methods for telemetry data evaluation include empirical and physiologically based approaches.[5] The latter aims to describe the interactions between various CV markers systemically and mechanistically, and, as such, is expected to be more reliable in between-species translation. However, the complexity of the CV system and the lack of data for thorough investigation of interspecies differences brings on additional assumptions, which makes the model-based translation challenging, especially for the first-in-class compounds.[6,7] Furthermore, development of the mechanistic models de novo usually requires extensive timeframes and lacks agility often sought for in early drug development.[8] On the other hand, empirical or pharmacokinetic (PK)/PD approaches, while mostly ignores the physiological context represents a flexible tool for quick and transparent fit-for-purpose analysis of telemetry data.[5]

The existing guidelines cover the quantitative evaluation of ECG parameters, such as PK/PD analyses for QT interval prolongation comparison and in vitro-driven systems biology models for ECG changes, and respective workflows more extensively compared to the modeling of the hemodynamic markers.[9,10] However, treatment-induced changes in hemodynamic measurements are equally widespread among the drugs.[11] Moreover, nonclinical telemetry study design and data patterns (i.e., continuous and frequent telemetry data recording; study separation into telemetry and PK data collection phases; substantial handling effects; and usually small sample size) distinguish the subsequent model-based analyses from its typical clinical counterparts.[5,12] Taken together, this makes the task of creating a strategy for the model-based assessment of CV safety on hemodynamic parameters highly relevant. In this tutorial, we provide a comprehensive workflow for nonclinical CV safety analysis of hemodynamic markers using empirical PK/PD models which covers all essential challenges in model development and interspecies translation. The workflow is comprised of four sequential steps, for each step, the main complications are explored, and solutions proposed, complemented by a reproducible example for an anti-inflammatory compound, which can be used for efficient model-based analyses of the CV safety of new drug candidates in R software[13] and Monolix environment.[14]

TYPES OF STUDIES AND DATA

Nonclinical studies for CV safety assessment are conducted primarily in rats, guinea pigs, dogs, and cynomolgus monkeys.[15] The choice of species depends on the stage of the project and an understanding on the potential mechanisms based on in vitro data generated in secondary/safety pharmacology screens along with primary pharmacology considerations. Study animals are equipped with telemetry recording devices and are given a range of doses of an investigated compound as well as placebo. CV measurements of interest, for example, arterial pressure, heart rate, and contractility of the ventricles, are recorded with a telemetry device over 24 h or more, to ensure continuous data collection throughout the full period of drug exposure.[16] Single or multiple doses can be administered throughout a study, single-dose studies possessing either parallel or crossover (partial or full Latin square) assignment design.[17] The latter is arguably more prevalent as it allows to minimize the number of animals required to conduct a study, although at the cost of study duration.[18,19] In contrast, multiple-dose studies are associated with the parallel assignment and are used for the assessment of delayed or cumulative CV effects. In all cases, the number of animals per study arm rarely exceeds eight animals.[19]

CV measurements tend to follow day and night cycles (i.e., circadian variations).[20] Furthermore, handling effects caused due to dosing and/or PK sampling often interfere with CV measurements, despite the efforts to minimize the impact during telemetry data recording.[21] Usually, only a single PK sample is collected from animals during the telemetry recordings, whereas detailed PK profiling is performed in a separate phase of the original telemetry study or in a satellite study on an entirely different set of animals.[12,22]

Sampling frequency of telemetry recordings may vary considerably depending on the telemetry device, animal, and type of CV measurement.[23] Ideally, the measurements are continuous, averaged over predefined time intervals for the subsequent analyses. Binning of data over large time intervals may obscure handling effects and circadian variations. Based on the history of our analyses, we recommend keeping it at or below 15 min for rats and use the specific timepoint ranges (e.g., every 30 min until the first 4 h after dosing, every 2 h between 4 and 12 h, and the subsequent measurements can be binned at 4-h intervals), chosen based on the PK profile of the compound for dogs. Other averaging strategies include using the data from the first 5 min of each hour[24] or dividing a time series into evenly spaced 30, 60, or 120 min intervals.[25]

At the preclinical stage of drug development, human PK data are not available. Therefore, conventional

allometric scaling from in vivo animal data, mechanistic (e.g., in vitro to in vivo extrapolation) and/or translational modeling methods like physiologically-based pharmacokinetic (PBPK) modeling are used to obtain predicted human PK parameters.[26,27]

OVERVIEW OF THE WORKFLOW

The framework of this tutorial is represented by a workflow for the model-based analyses of nonclinical telemetry studies of CV safety pharmacology, which uses a sequential approach and consists of four consecutive steps: (1) PK modeling, (2) quantification of circadian variations and handling effects, (3) drug effect modeling, and (4) animal-to-human translation (Figure 1). The purpose of the first step is to develop an optimal PK model for the compound in question. Circadian variations and the effects from animal handling are the subjects of the second step. The functional relationship between drug exposure and PD response is quantified with a model fit to the PD data, conditional on the parameters estimated on the previous steps of the workflow. Finally, the animal PK model is replaced by the human PK model, and the biomarker response is evaluated in the range of clinically relevant exposures. The choice of the sequential approach over the simultaneous one is substantiated by the small number of animals coupled with the complex patterns in the data (discussed below) and sparce PK sampling.[28] Furthermore, steps 2 to 4 are conducted independently for each type of measurement, whereas PK model structure and parameters remain the same.

All steps are preceded by an exploratory analysis of the available measurements which aims to decompose observed data to provide qualitative priors for the subsequent mathematical modeling. The main set of the exploratory plots for PK includes visualization of time series of drug concentration measurements, by animal and aggregated, as well as different metrics of exposure (average, peak and trough concentrations, and area under curve [AUC]) across doses, study phases, or studies (Figure 2a–c). Thorough telemetry data exploration requires visualization of unadjusted, baseline-adjusted, and baseline-adjusted placebo-corrected time profiles of each biomarker, aggregated by study arm (Figure 2d–f).

Each stage of the workflow can be associated with multiple challenges. We attempted to summarize them all, together with respective solutions, in a comprehensive and organized manner (subsequent sections; Table 1), to provide a practical guide for the pharmacometricians and boost the efficiency of such analyses. Mathematical models applied for the task are based on the nonlinear systems of ordinary differential equations (ODEs), their development relies on numerical methods of solving the direct and inverse problems which are well-established and implemented in various software, such as MATLAB,[29] NONMEM,[30] Monolix,[14] R packages,[13] etc.[31] As we are utilizing conventional metrics and criteria for model development and selection, we will not be covering them in this tutorial and will refer the reader to the respective guides and reviews.[32,33]

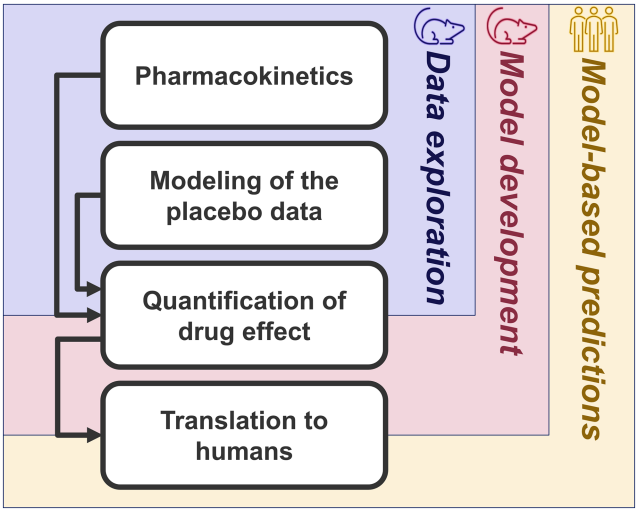

FIGURE 1 Sequential workflow of model-based telemetry data analyses.

PK MODELING

Compartmental modeling of individual PK data has been extensively studied and utilized in drug development for over 40 years.[34,35] It works equally well for preclinical and clinical data alike.[36] One of the typical examples of such models (2-compartment with first-order absorption) is represented by ODEs in Equations 1–3:

$$\frac{dA_d}{dt} = -k_a * A_d, \tag{1}$$

$$\frac{dA_c}{dt} = k_a * A_d - \frac{CL}{V_c} * A_c + \frac{Q}{V_p} * A_p - \frac{Q}{V_c} * A_c, \tag{2}$$

$$\frac{dA_p}{dt} = \frac{Q}{V_c} * A_c - \frac{Q}{V_p} * A_p, \tag{3}$$

$$C_c = \frac{A_c}{V_c}, \tag{4}$$

where A_d is the amount of drug in dosing compartment; A_c is the amount of a drug in central compartment; A_p

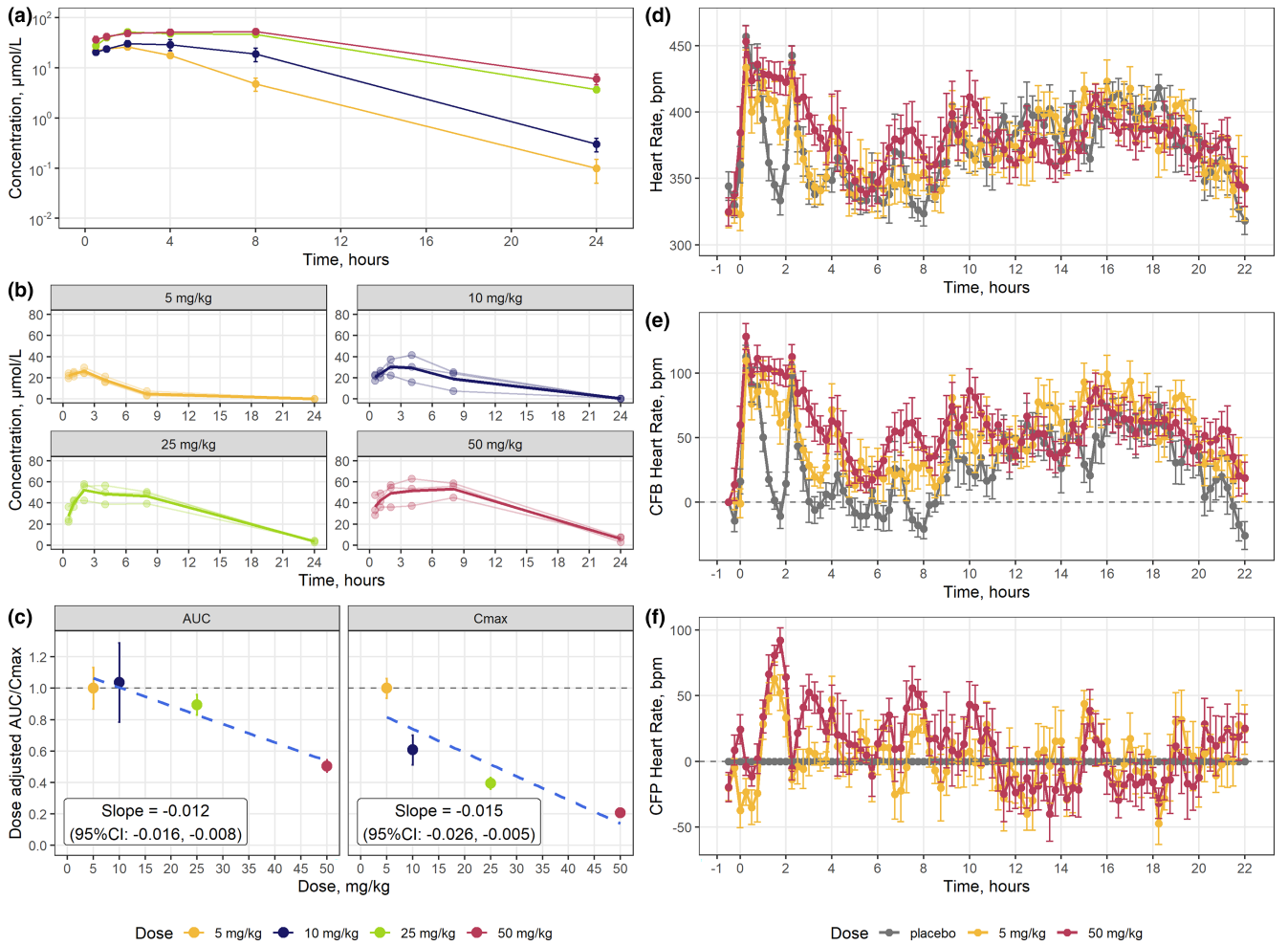

FIGURE 2 Rat telemetry data visualization. (a) Semilogarithmic plot of plasma PK after single dose administered by oral gavage; dots with error bars—mean ± SE. (b) PK profiles stratified by dose; dots and thin lines—individual data, thick lines—mean trends. (c) Dose-adjusted AUC and C_{max} versus dose; dots with error bars—mean ± SE, dashed blue line—linear regression. Change over time in heart rate (d), baseline-adjusted (e), baseline- and placebo-adjusted (f); dots with error bars—mean ± SE. AUC, area under the curve; CFB, change from baseline; CFP, change from placebo; C_{max}, maximum plasma concentration; PK, pharmacokinetic.

is the amount of a drug in peripheral compartment; k_a is the absorption rate constant; V_c is the central volume of distribution; V_p is the peripheral volume of distribution; CL is the linear clearance; Q is the intercompartment clearance; and C_c is drug concentration in central compartment.

The choice of the structural model is driven primarily by the available PK measurements and prior knowledge of the physiochemical and PK properties of the compound.[37] The doses, usually administered orally in CV studies, are designed to achieve a wide range of plasma and tissue exposures that enables meaningful detection of CV signals. High doses (supratherapeutic) can often lead to nonlinearities in absorption and/or systemic clearance, which can be detected early by plotting various dose-adjusted measures of exposure against the dose and estimating the significance of the slope in the respective linear relationship (Figure 2c). If the slope parameter is not statistically significant (i.e., 95% confidence interval includes zero), the exposure increases in proportion to the dose, and the structural model can be used as is. Otherwise, some modifications may be required, depending on the observed trends.

A decrease in the average drug concentration or AUC measured throughout the dosing period relative to the dose (Figure 2c, left panel) can be captured either explicitly, by introducing dose-dependent bioavailability (Equation 5), or more physiologically, by implementing additional rate to Equation 1 (Equation 6):

$$A_d(t_{dose}) = \mathrm{DOSE} * \left(1 - \frac{I_{max} * \mathrm{DOSE}}{(\mathrm{DOSE} + \mathrm{ID}_{50})}\right), \quad (5)$$

$$\frac{dA_d}{dt} = -k_a * A_d - k_{ex} * A_d, \quad (6)$$

TABLE 1 Challenging patterns in the data and respective modeling solutions.

Data pattern	Exploratory plot	Modeling solution
PK modeling		
Exposure is not dose-proportional (lower than expected)	Dose-adjusted exposure versus dose (Figure 2c, left panel)	• Dose-dependent bioavailability: $A_d(t_{dose}) = DOSE * \left(1 - \frac{I_{max} * DOSE}{(DOSE + ID_{50})}\right)$ • Excretion from the intestine: $\frac{dA_d}{dt} = -k_a * A_d - k_{ex} * A_d$
Exposure is not dose-proportional (higher than expected) or two phases of elimination	Dose-adjusted exposure versus dose (Figure 2c, left panel)	• Combination of linear and saturable elimination: $\frac{dA_c}{dt} = k_a * A_d - CL * C_c - \frac{V_m * C_c}{(K_m + C_c)}$
C_{max} is not dose-proportional (lower than expected)	Dose-adjusted C_{max} versus dose (Figure 2c, right panel)	• Saturable absorption: $\frac{dA_d}{dt} = -\frac{V_a * A_d}{(IA_{50} + A_d)}$
C_{max} is not dose-proportional (higher than expected)	Dose-adjusted C_{max} versus dose (Figure 2c, right panel)	• Saturable excretion from the intestine $\frac{dA_d}{dt} = -k_a * A_d - \frac{V_{ex} * A_d}{(IA_{50} + A_d)}$
Notable between-study variability	Aggregated PK profiles stratified by study (Figure 3c)	• Introduce study as a categorical covariate on one or more PK parameters
Notable between-animal variability in PK data	Individual PK profiles (Figure 2b)	• Introduce random effects on one or more PK parameters
Marked differences in PK between two phases of a single study	Individual or aggregated PK profiles stratified by study phases	• For parallel assignment studies: introduce study phase as a categorical covariate on one or more PK parameters • For crossover studies: introduce between-occasional variability
Handling effect and circadian variations modeling		
Interspecies differences in circadian variations	Time course of CV marker in placebo arm (Figure 2d,e)	• Use single cosine function as a circadian variation model: $CR(t) = k_w * \cos\left(\frac{2\pi(t - k_0)}{k_{period}}\right)$ • Add additional cosine function for intraday harmonic variations, if needed
Observed handling effects associated with feeding and blood sampling	Time course of CV marker in placebo arm (Figure 2d,e)	• Test different functions to describe handling effect: Exponential function: $HE(t) = \begin{cases} M_{he} * \exp\left(-k_{he} * \left(t - t_{event}\right)\right), t \geq t_{event} \\ 0, t < t_{event} \end{cases}$ Biexponential function: $HE(t) = \begin{cases} M_{he} * \left(\exp\left(-k_{he} * \left(t - t_{event}\right)\right) - \exp\left(-k_a * \left(t - t_{event}\right)\right)\right), t \geq t_{event} \\ 0, t < t_{event} \end{cases}$ Gaussian function: $HE(t) = \begin{cases} M_{he} * \exp\left(-\frac{(t - k_1)^2}{2 * k_2^2}\right), t \geq t_{event} \\ 0, t < t_{event} \end{cases}$
Different shapes between handling effects within single observation period		• Use different magnitudes for different handling effects
Apparent threshold in the magnitude handling effect		• Introduce physiologically based maximum for selected markers

(Continues)

TABLE 1 (Continued)

Data pattern	Exploratory plot	Modeling solution
Drug effect modeling		
Unknown functional relationship between PK and drug effect	Time course of CV marker in treatment arms (Figure 2d,e)	• Test different functional relationships: Linear model: $\mathrm{Eff} = k_{\mathrm{eff}} * C_{\mathrm{c}}$ Emax-model: $\mathrm{Eff} = \frac{E_{\max} * C_{\mathrm{c}}^{\gamma}}{\mathrm{EC}_{50}^{\gamma} + C_{\mathrm{c}}^{\gamma}}$
Handling effect interferes with the drug effect	Baseline-adjusted placebo-corrected time course of CV marker in treatment arms (Figure 2f)	• Exclude handling effect from the drug effect modeling • Simultaneous placebo and drug effect parametrization with physiologically based maximum threshold for a biomarker
Delay in the drug effect	Concentration-effect plot linked by the timepoints (Figure 5b)	• Introduce biophase effect compartment: $\frac{dC_{\mathrm{e}}}{dt} = k_{\mathrm{e}} * \left(C_{\mathrm{c}} - C_{\mathrm{e}}\right)$ • Replace explicit function with a turn-over equation for a biomarker
Apparent decrease of the drug effect over time	Observed PD versus predicted PK time profiles (Figure 5d)	• Introduce time-dependent adaptation function (linear, exponential, Michaelis–Menten or Hill functions): $\mathrm{Eff}_{\mathrm{adapt}} = \mathrm{Eff} * \left(1 - \frac{t^{\tau}}{\mathrm{ET}_{50}^{\tau} + t^{\tau}}\right)$

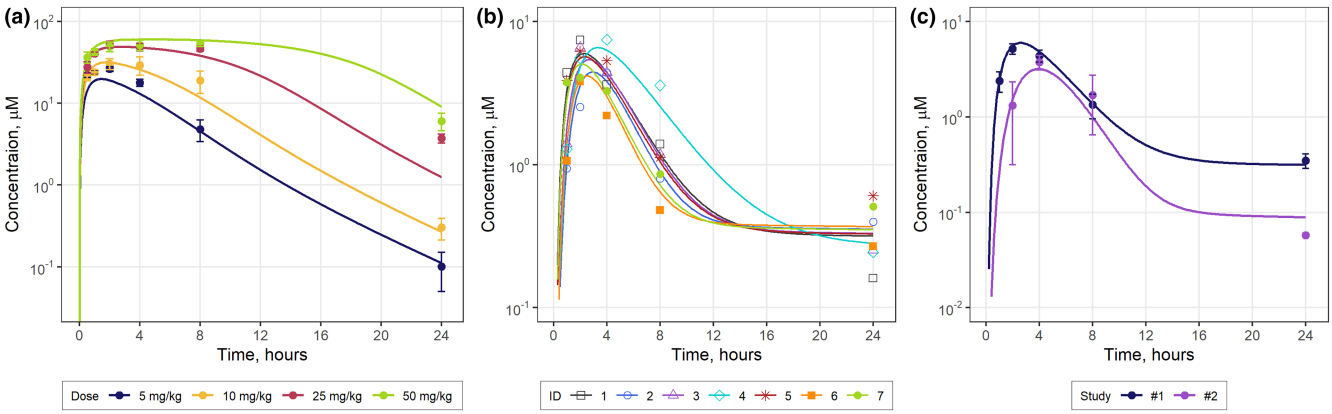

FIGURE 3 Time profiles of observed and predicted PK in rats. (a) PK model with saturable absorption; dots with error bars—mean ± SE, curves—model predictions. (b) PK model with random effects; dots—individual observations, curves—individual predictions, ID—animal identifier. (c) PK model with study as a covariate on clearance and intercompartmental flux; dots with error bars—mean ± SE, curves—model predictions. PK, pharmacokinetic.

where A_{d} is the amount of drug in dosing compartment; DOSE is the dose of the compound; $I_{\max}$ is the maximum effect ($I_{\max} \in [0, 1]$); ID_{50} is the dose corresponding to the 50% of the maximum effect; k_{a} is the absorption rate constant; and k_{ex} is the excretion rate constant.

Likewise, if the dose-adjusted maximum concentration ($C_{\max}$) falls with dose (Figure 2c, right panel), Equation 1 is to be changed to include saturable absorption (Figure 3a, Equation 7):

$$\frac{dA_d}{dt} = -\frac{V_a * A_d}{\left(IA_{50} + A_d\right)}, \tag{7}$$

where V_{a} is the maximum absorption rate; IA_{50} is the amount of the drug required to achieve 50% of the maximum absorption rate; and A_{d} is the amount of drug in dosing compartment.

In some cases, a reverse trend can be observed—when AUC or $C_{\max}$ is more than dose proportional. Such behavior AUC can be captured by using second, saturable clearance in Equation 2 (Equation 8):

$$\frac{dA_{\mathrm{c}}}{dt} = k_{\mathrm{a}} * A_{\mathrm{d}} - \mathrm{CL} * C_{\mathrm{c}} - \frac{V_{\mathrm{m}} * C_{\mathrm{c}}}{\left(K_{\mathrm{m}} + C_{\mathrm{c}}\right)}, \tag{8}$$

where A_c is the amount of drug in central compartment; k_a is the absorption rate constant; A_d is the amount of drug in dosing compartment; CL is the linear clearance; V_m is the maximum clearance (saturable); K_m is the drug concentration required to achieve 50% of the maximum clearance (saturable); and C_c is drug concentration in central compartment.

The increasing behavior of C_{max} with dose can be observed due to saturation in presystemic metabolism and can be described either by Equation 8 or by using saturable excretion from the intestine (Equation 9):

$$\frac{dA_d}{dt} = -k_a * A_d - \frac{V_{ex} * A_d}{(IA_{50} + A_d)}, \quad (9)$$

where A_d is the amount of drug in dosing compartment; k_a is the absorption rate constant; V_{ex} is the maximum excretion rate; and IA_{50} is the amount of the drug required to achieve 50% of the maximum absorption rate.

Other complications, such as enterohepatic circulation, can be present in the data as well and should be accounted for in the model.[38] Such cases are unique and require a tailored approach for both preclinical modeling and subsequent translation to humans.

One of the main aims of the population PK modeling analyses is to distinguish between-subject variability from the residual error and attempt to partially explain it with individual properties, that is, the covariates.[39] However, estimating random effects in telemetry studies with a small number of animals will inevitably result in overparameterization and shrinkage beyond any acceptable range.[32] Nevertheless, if notable between-animal variability, characterized by coefficient of variation exceeding 50% for the majority of the timepoints, is present in the data (Figure 3b**)**, individual parameters can be estimated and fixed for the subsequent modeling steps to capture exposure-response relationship more accurately. In extreme cases of variability in crossover design, additional levels of hierarchies in random effects can be included (i.e., occasions). Inference regarding the distribution of random effects is limited because random effects are estimated from a small number of animals. If PK and PD measurements are made from different animals, instead of using individual PK predictions for animals from telemetry study, population parameters from PK model are used for subsequent PD modeling.

Consequently, covariate search is also usually omitted. However, if a pooled analysis is being performed using the data from several telemetry studies, animal age, model, or the study itself can be used as a factor affecting parameter estimates, regardless of the presence of random effects (Figure 3c).

HANDLING EFFECT AND CIRCADIAN VARIATIONS

CV markers, such as heart rate, blood pressure, and cardiac contractility are susceptible to natural diurnal oscillations, specific for each type of measurement and animal.[40] Visualization of the continuous hemodynamic telemetry data collected within 24 h clearly illustrates these trends (Figure 2d,e).

Standard model-based approach to describe such data is to apply trigonometric functions that oscillate with specific amplitude and phase over a certain time period[41]:

$$CR(t) = k_w * \cos\left(\frac{2\pi(t - k_0)}{k_{period}}\right), \quad (10)$$

where $CR(t)$ is the time-dependent function to describe circadian variations; k_w is the amplitude of oscillations; k_0 is the horizontal displacement; and k_{period} is the oscillations period.

Consequently, change over time in a biomarker in placebo cohort can be described by the following explicit function (Figure 4a,b):

$$BIOM(t) = BIOM_{BL} + CR(t), \quad (11)$$

where $BIOM_{BL}$ is the baseline value of a biomarker.

Ideally, parameters reflecting amplitude, horizontal displacement, and period of oscillations should be estimated against the data in each specific modeling case. In practice, as the data are typically available in the range of 1 to 2 days and are always limited, by default, k_{period} is fixed at 24 h to ease the computation of optimal parameter values, considering that physiological functions have 24 h rhythm.[42] Furthermore, if the k_0 parameter is not identifiable (95% confidence interval for the point estimate includes zero), it can be set to zero as, apparently, no shift in the oscillation phase is required to describe the observed data.

On occasions when rich sampling is available, more than one trigonometric function can be parametrized (Figure 4c):

$$BIOM(t) = BIOM_{BL} + CR_1(t) + \ \ldots \ + CR_i(t), \quad (12)$$

where $CR_1(t) \ldots CR_i(t)$ are structurally identical to Equation 11, but with unique sets of parameters.

However, based on our experience and historical data, identifying more than two cosine functions is challenging.[6,7,43] Furthermore, the second circadian variation function has notably shorter period (~2 h) and amplitude (~12%), contributing little toward the accuracy of further predictions and, arguably, could be ignored.

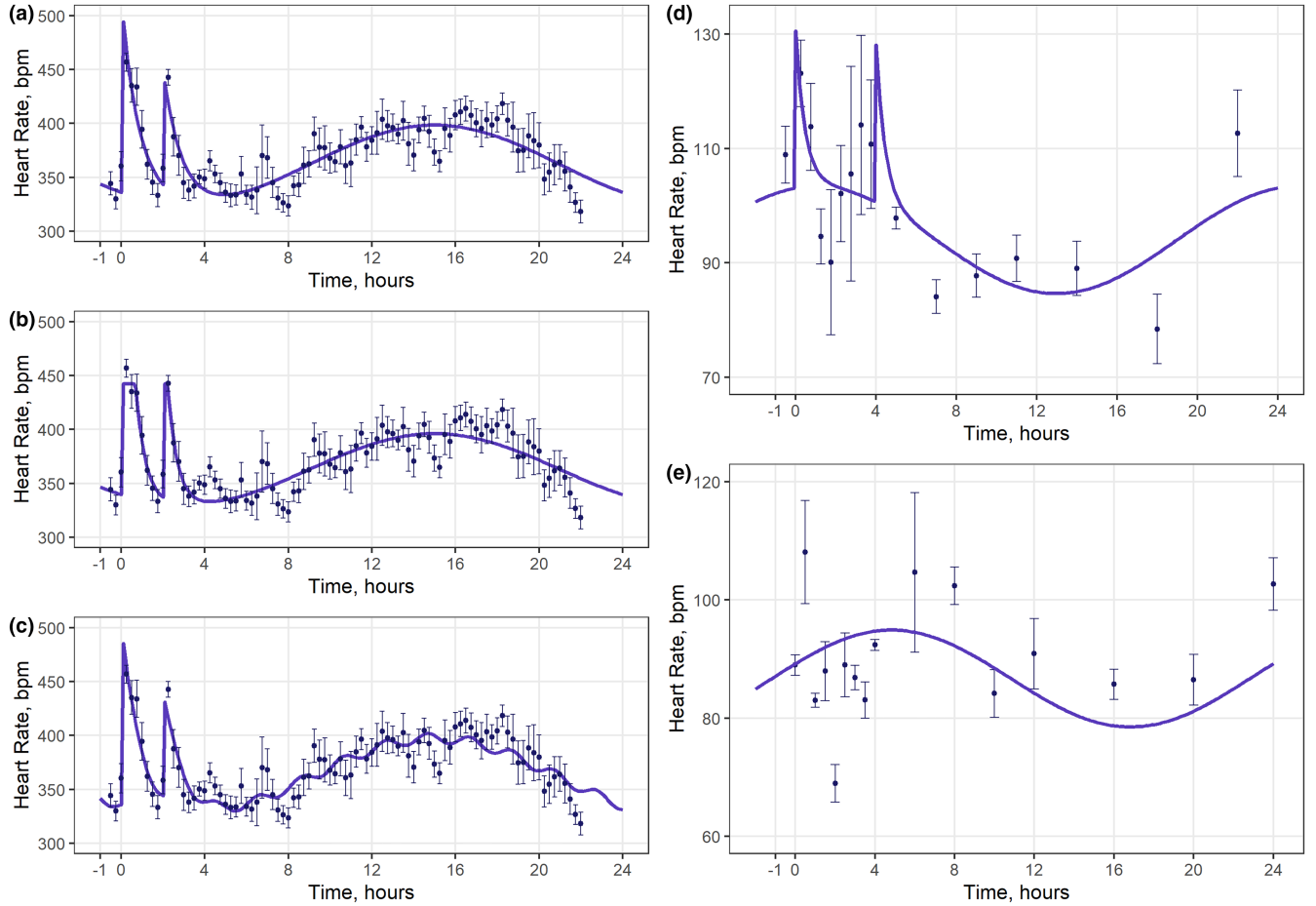

FIGURE 4 Observed and predicted heart rate in rats (a–c) and dogs (d–e) in control arms. (a) Single cosine function, different magnitude for each handling effect without physiological threshold. (b) Same as panel a, with physiological threshold. (c) Same as panel a, with two cosine functions. (d) Single cosine function, same magnitude for each handling effect without physiological threshold. (e) Same as panel d, no handling effects. Dots with error bars—mean ± SE, curves—model predictions.

The second major complication in telemetry data modeling is the biomarker signals associated with human-animal interactions. These handling effects are typically observed at two instances: drug administration (at timepoint zero) and blood sample collection for PK measurements (usually at 4 h after dose). The procedures are triggering stress-like reactions provoking sharp changes in CV markers, which can mask a treatment effect to a significant degree, especially if the PD response is most profound within the first 8 h after dose[21] (Figure 2d,e).

There are many ways to reproduce handling effects in a mathematical model, the choice depends primarily on the richness of sampling and the shape of the data. Arguably the most common option is to use exponential function[6,44]:

$$\mathrm{HE}(t)=\begin{cases} M_{\mathrm{he}} * \exp\left(-k_{\mathrm{he}} * \left(t-t_{\mathrm{event}}\right)\right), t \geq t_{\mathrm{event}} \\ 0, t < t_{\mathrm{event}} \end{cases} \quad (13)$$

where M_{he} is the magnitude of a handling effect; k_{he} is the reduction rate of a handling effect; and t_{event} is the time of onset of a handling effect.

Then, Equation 11 can be modified in the following way:

$$\mathrm{BIOM}(t) = \mathrm{BIOM}_{\mathrm{BL}} + \mathrm{CR}(t) + \sum_{h=1}^{H} HE_h(t) \quad (14)$$

where H is the number of manipulations with animals that provoked handling effect.

The disadvantage of such a function is the immediate onset of the handling effect, governed by the M_{he} parameter. It ignores the initial amplification in the CV marker dynamics and theoretically inevitably overpredicts the effect between the last observation before the onset of the effect and the first observation after. Moreover, a caution should be exercised to avoid overlapping the time of the last observation before the handling effect and the onset of the latter, represented by t_{event} in the modeling dataset.

To account for these limitations, more complex solutions can be utilized, such as biexponential function (Equation 15) or Gaussian function (Equation 16):

$$\mathrm{HE}(t)=\begin{cases} M_{\mathrm{he}}*\left(\exp\left(-k_{\mathrm{he}}*\left(t-t_{\mathrm{event}}\right)\right)-\exp\left(-k_{\mathrm{a}}*\left(t-t_{\mathrm{event}}\right)\right)\right), t\geq t_{\mathrm{event}} \\ 0, t<t_{\mathrm{event}} \end{cases} \quad (15)$$

$$\mathrm{HE}(t)=\begin{cases} M_{\mathrm{he}}*\exp\left(-\frac{\left(t-k_1\right)^2}{2*{k_2}^2}\right), t\geq t_{\mathrm{event}} \\ 0, t<t_{\mathrm{event}} \end{cases}, \quad (16)$$

where M_{he} is the magnitude of a handling effect; k_{he} is the reduction rate of a handling effect; k_{a} is the rate of appearance of a handling effect; k_1 is the position of the center of the peak of a handling effect; k_2 is its standard deviation; and t_{event} is the time of onset of a handling effect.

These functions allow to capture the initial phase in a CV marker response provoked by the animal handling. However, the data rarely allows us to identify the additional parameter present in the equations. As such, the simplified solution represented by Equation 13 is usually sufficient.

As mentioned above, only two handling effects are usually observed within a dosing period (i.e., $H = 2$ in Equation 14). Both can be characterized by a unique set of parameters and functions. In practice, varying the magnitude of handling effects (M_{he}) or even using the same set of parameters is enough to achieve proper parametrization and adequate description of the observed data.

One more potential complication, in addition associated with the subsequent modeling of the pharmacological effect of the drug, is the physiological threshold of a biomarker response. In certain instances, the handling effects are clearly observed in placebo-treated animals but are completely missing in the active arms of a study, as shown in Figure 2d,e. Furthermore, the magnitude of handling effects concurs with the maximum effect of the drug. Such behavior in biomarker dynamics is hypothesized to be associated with complex physiological feedbacks preventing hemodynamic markers from reaching life-incompatible values.[45] As such feedbacks are out of scope of an empirical PK/PD model, the simplest solution is to explicitly indicate physiological limits in the model structure:

$$\mathrm{BIOM}(t)=\begin{cases} \mathrm{BIOM_{BL}}+\mathrm{CR}(t)+\mathrm{HE(t)}, \text{for } \mathrm{BIOM_{min}}<\mathrm{BIOM}(t)<\mathrm{BIOM_{max}} \\ \mathrm{BIOM_{max}}, \text{for } \mathrm{BIOM}(t)\geq\mathrm{BIOM_{max}} \\ \mathrm{BIOM_{min}}, \text{for } \mathrm{BIOM}(t)\leq\mathrm{BIOM_{min}} \end{cases} \quad (17)$$

where $\mathrm{BIOM_{max}}$ and $\mathrm{BIOM_{min}}$ are maximum and minimum thresholds of a biomarker, respectively.

Random effects are usually not considered for the parameters in the equations above. Furthermore, as all animals within a study live in the same conditions and are treated equally, there are no prerequisites to consider between-animal variability in circadian oscillations and handling effects. Baseline levels of biomarkers can be an exception, although contemplating the same challenges of an extremely small sample, as in the case with PK submodel.

IMPLEMENTATION OF THE DRUG EFFECT

The final part of the animal model development is the quantification of the safety effect of a drug by joining the PK, circadian variations, and handling effects submodels (parameters estimated in these submodels are fixed), and using the data from active arms of a telemetry study to parametrize the relationship between unbound drug concentration and biomarker response. It poses four main challenges: (1) the choice of functional relationship, (2) interference from the handling effects, (3) delay in the drug effect, and (4) apparent decrease of the drug effect over time.

For the first issue, two fundamental types of dependencies are considered: with and without saturation. By default, the former is represented by a linear equation, the latter—by maximum effect (E_{max}) function[41]:

$$\mathrm{Eff}(t)=k_{\mathrm{eff}}*C_{\mathrm{c}}(t), \quad (18)$$

$$\mathrm{Eff}(t)=\frac{E_{\mathrm{max}}*C_c(t)^{\gamma}}{EC_{50}{}^{\gamma}+C_c(t)^{\gamma}}, \quad (19)$$

where Eff represents the drug effect function; k_{eff} is the slope of a linear function; E_{max} is the maximum effect; $\mathrm{EC_{50}}$ is the drug concentration required to achieve 50% of the maximum effect; γ if the Hill coefficient; and C_{c} represents the drug concentration in central compartment.

As such, treatment-mediated changes of a biomarker can be represented by the following equation:

$$\mathrm{BIOM}(t)=\left(\mathrm{BIOM_{BL}}+\mathrm{CR}(t)+\sum_{h=1}^{H}\mathrm{HE}_h(t)\right)*(1\pm\mathrm{Eff}(t)). \quad (20)$$

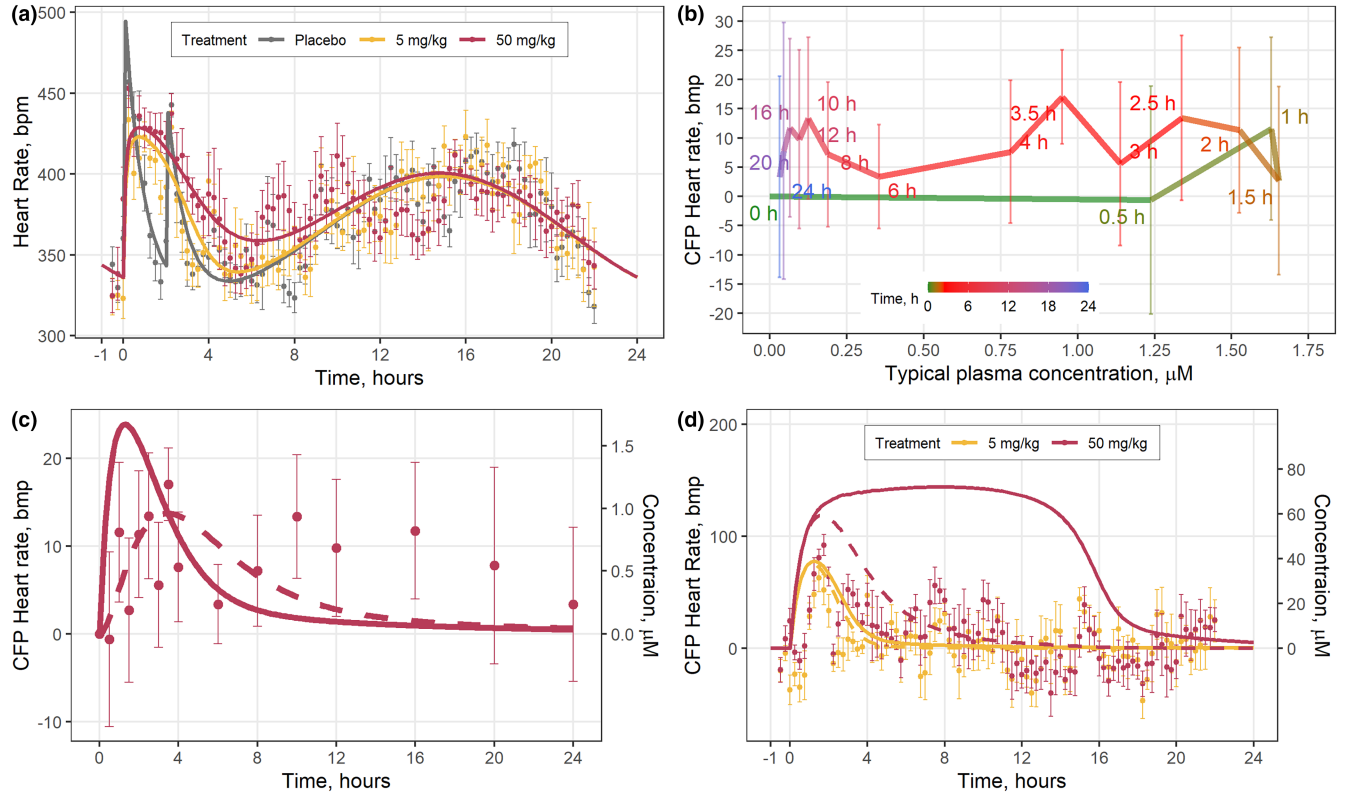

FIGURE 5 Animal toxicodynamic data patterns and associated modeling solutions. (a) Observed and predicted response in placebo and treated cohorts with handling effect excluded from the drug effect model; dots with error bars—mean ± SE, curves—model predictions. (b) Hysteresis plot for mean baseline- and placebo-adjusted heart rate against the simulated PK; error bars—95% CI. Change over time in baseline- and placebo-adjusted heart rate against the simulated PK; dots with error bars—observed mean heart rate ± SE, for (c) solid curve—predicted PK in central compartment, dashed curve—predicted PK in biophase compartment, for (d) solid curve—predicted PK without adaptation function, dashed curve—predicted PK with adaptation function. CFP, change from placebo; CI, confidence interval; PK, pharmacokinetic.

The product is used instead of the sum or difference to avoid potential conflicts in subsequent translation of the effect to humans.

Using the E_{max} equation is safer for the extrapolations and better corresponds to the physiology but requires the available range of doses to cover both the slope and the plateau of the safety signal. In addition, it may come in conflict with handling effects which can obscure the drug-induced changes, especially when they are close to the physiological limit (Figure 2f). If physiological limits are explicitly implemented into the model (Equation 17) and estimated at the previous step of the workflow, quantification of a separate parameter of maximum drug effect becomes impossible, narrowing the choice of equation to the ones without saturation. Alternatively, the data points within the handling effect intervals can be disregarded for the parameter estimation procedure (Figure 5a**)**. Both options are flawed, however, the first one can be considered more conservative for the animal-to-human translation, as no saturation in safety effect will be predicted for humans in such a case.

It can be noted that Equation 20 is a direct response model, and no differential equations are used to describe biomarker response. Meanwhile, the safety signals on CV markers often comes with a delay relative to the plasma drug concentration, that is, hysteresis. Hysteresis can be observed following a delay in distribution of a drug into the site of effect, sensitization of receptors, the formation of active metabolites, etc., and is usually detected by matching biomarker data with the drug concentration in a loop-like plot.[46–48] This plot requires simultaneous measurements of PK and biomarker data, which is not the case for telemetry studies. However, as a sequential modeling approach is utilized in this workflow, simulated PK concentrations can be used instead of the actual observed ones (Figure 5b**)**. Nevertheless, it is always advisable to evaluate the presence of temporal differences between PK and toxicokinetic response by replacing the explicit function with a turn-over equation for a biomarker or introducing biophase effect compartment and attempting to identify k_e[46]:

$$\frac{dC_e}{dt} = k_e * (C_c - C_e), \tag{21}$$

where C_e represents drug concentration in the biophase effect compartment; C_c represents drug concentration in the central compartment; k_e is the transition rate between central and biophase compartments so that Cc in Equations 18 and 19 is replaced by Ce (Figure 5c). It is important to note that the PK submodel equations are not affected by the biophase.

The opposite situation is observed when the safety signal diminishes over time while drug exposure persists in the circulation, as illustrated by Figure 5d. This phenomenon can be explained by the adaptation of the CV system to drug-induced stimuli and described by introducing time-dependent term into the drug effect equation:

$$\mathrm{Eff}_{\mathrm{adapt}}(t) = \mathrm{Eff}(t) * \left(1 - \frac{t^{\tau}}{ET_{50}{}^{\tau} + t^{\tau}}\right), \qquad (22)$$

where $\mathrm{Eff}_{\mathrm{adapt}}$ represents the drug effect function after adaptation; Eff is the drug effect function; ET_{50} is the time required to achieve 50% of the maximum effect; and τ is the Hill coefficient.

It is plausible to introduce mixed effects to drug effect parameters, such as k_{eff} (Equation 18) or $E_{\max}$ (Equation 19). Introducing random effects on EC_{50} (Equation 19) is not recommended, as under limited number of doses and associated concentrations, $E_{\max}$ is expected to strongly correlate with EC_{50}, which results in overestimation of standard deviation of the random effects for the parameters. Achieving adequate description of the treatment-mediated changes in CV markers considering the aspects outlined above concludes the model development and precludes animal-to-human translation.

CHALLENGES IN TRANSLATION

Translation modeling of the potential safety profile is a straightforward process, schematically outlined in Figure 6. In short, the animal PK model is replaced by a human PK model (including fraction unbound in plasma), developed from preclinical data and/or PBPK models.[26,49,50] Circadian variations and handling effects are then excluded from human model development, whereas human baseline levels of the biomarkers are imputed (Table 2). Physiological limits and loss of effect over time are also excluded from the model structure. Biophase (i.e., the delay in a drug effect), if parametrized during animal model development, is kept in the human model.

This translational approach assumes that the safety profile is mostly off-target and the mechanisms causing undesirable effects of a drug on CV markers are similar between humans and animals. Where possible, an

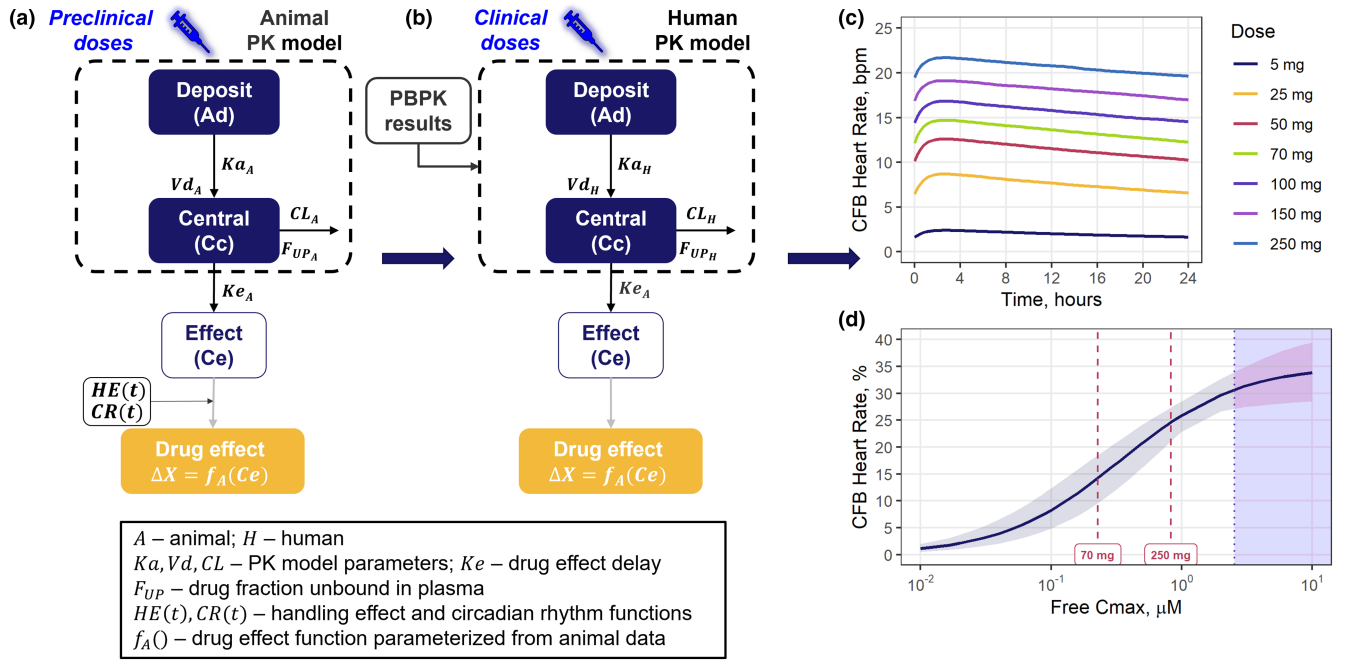

FIGURE 6 Schematic representation of the animal-to-human translation workflow. (a) Animal model schematics. (b) Human model schematics. (c) The upper limit of 95% prediction interval for the absolute change from baseline in human heart rate under treatment at steady state; curves—model predictions. (d) Exposure-response plot for the relative change from baseline in human heart rate; solid curve with shaded area—predicted mean with 95% prediction interval, red dashed lines—exposure corresponding to the 97.5% percentile of the $C_{\max}$ prediction distribution at selected doses, shaded region—exposure exceeding maximum concentrations observed in animals (i.e., extrapolation). CFB, change from baseline.

TABLE 2 Baseline hemodynamic parameters and circadian variation parameters for animals and human.

Biomarker	Parameter	Rats	Dogs	Humans
HR	k_w, bpm	37.81 (±4.4)	9 (±2.2)	
	k_0, h	15.12 (±0.5)	5.13 (±4.6)	
	$BIOM_{BL}$, bpm	333.91 (±24.4)	87.05 (±6.7)	[60; 100][54]
MAP	k_w, mmHg		8.04[a]	
	k_0, h		2.32[a]	
	$BIOM_{BL}$, mmHg		112.3[a]	[95; 104][b]
SBP	k_w, mmHg	4.67[a]	4.66 (±1.2)	
	k_0, h	16.6[a]	9.4 (±12.2)	
	$BIOM_{BL}$, mmHg	132[a]	140.67 (±10.8)	[120; 129][55]
DBP	k_w, mmHg	4.06[a]	3.94 (±1.8)	
	k_0, h	16[a]	7.8 (±8.6)	
	$BIOM_{BL}$, mmHg	92.9[a]	88.9 (±8.9)	[70; 79][55]
CC (dP/dt)	k_w, mmHg/s		415.78 (±238.85)	
	k_0, h		2.09 (±0.94)	
	$BIOM_{BL}$, mmHg/s		4197.9 (±310.4)	>1200[56]

Note: Parameter values for rats and dogs are taken from seven internal telemetry analyses if not indicated otherwise. Numbers are mean ± SE (animals) or healthy range (humans). Empty cells—data not available. HR—heart rate; MAP—mean arterial pressure; SBP—systolic arterial pressure; DBP—diastolic arterial pressure; CC—cardiac contractility.

[a]Value from a single telemetry study.

[b]Calculated by averaging corresponding SBP and DBP.

integrated assessment on translation between preclinical species and humans is done based on the data generated from in vitro molecular screens, such as Ion channels (hERG, Nav1.5, Cav1.2, IKs, and ITO), phenotypic screens (for detecting changes in cardiac contractility—hiPS-CM FliPR), secondary pharmacology panels (e.g., VEGFR2 inhibition leads to blood pressure elevation), and appropriate scaling factors where available are applied to PK/PD models.[51] On occasions where the mechanisms are not understood, assuming similarity between species is reasonable (i.e., fit for purpose at this stage of drug development), although it ignores between-species differences in contribution of various regulatory systems toward the CV homeostasis and is the major limitation of the analysis. To partially compensate for the lack of physiological insight in the translation, random effects are applied on human PK parameters. A typical approach is to apply variability corresponding to the coefficient of variation of 25% to 40% on volume of distribution and clearance in a one-compartment model, with inter-parameter covariance of 30%.[52] Furthermore, if random effects were introduced at the previous step of the workflow, they will also contribute to the variability in the predicted safety response.

The ultimate goal of these predictions is to evaluate the CV safety of the doses selected for the first-in-human (FIH) trials. We propose to make decisions based on the upper bound of the 95% prediction interval for the peak change from baseline in a biomarker level under treatment at steady-state (Figure 6c,d). To calculate prediction interval, both uncertainty and variability in the model parameters should be taken into account.[53] The former can only be included for the drug effect parameters from the previous step of the analysis workflow. It is advisable to sample at least 1000 random effects (i.e., subjects) per 1000 population parameters (i.e., populations) per dosing scenario.[39] Steady-state is considered to be achieved when peak levels of the drug and the biomarker are not changing between the dosing periods.

To fully grasp the theoretical exposure-response relationship, peak biomarker changes can be plotted against the 97.5 percentile of the prediction distribution of free maximum drug concentration at steady-state following simulations with a wide array of doses (Figure 6d). Then, the conclusions can be drawn on the therapeutic window itself after establishing the acceptable thresholds for the hemodynamic measurements of interest, although caution in inference should be exercised for the drug concentrations exceeding those observed in animals.

The following decision criteria were developed for heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and cardiac contractility (CC). According to the National Institutes of Health, a normal resting HR for adult humans ranges between 60 and 100 bpm.[54] Exceeding these limits

would indicate bradycardia and tachycardia, respectively. Therefore, taking 80 bpm HR as a reference, treatment-induced changes in HR should not surpass ±20 bpm, or ±25% from baseline. This threshold is more conservative than that proposed by Leishman et al. where the magnitude of effect in humans was suggested by 40 bpm HR increase or HR reaching above 110 bpm.[19] Likewise, healthy SBP and DBP values correspond to less than 130 and less than 80 mmHg, whereas respective measurements above 140 and 90 mmHg are considered hypertension.[55] As such, we propose to consider SBP changes exceeding ±10 mmHg (±8%) and DBP changes exceeding ±10 mmHg (±12.5%) as meaningful in a clinical setting. Consequently, MAP should not change above or below ±10 mmHg (±10%) for a dosing regimen to be considered as safe. This threshold is also more conservative than that assumed in Leishman et al.[19] According to the reference values for dP/dt (a measure of CC)—dP/dt greater than 1200 mmHg/s is considered healthy. Reduced contractility is diagnosed if dP/dt less than 800 mmHg/s, that is, a decrease of CC within 34% is considered to be acceptable.[56] The decision criteria discussed provides a threshold for meaningful (clinically and reliably measurable) changes that may need clinical attention. Although it should be noted that much smaller/larger changes in CV parameters may or may not have impact on cardiac health depending on the patient population and compensatory mechanisms. Hence, the clinical implications of changes of any magnitude should always be interpreted in context of the patient population, underlying comorbidities, comedications, etc.

REPRODUCIBLE EXAMPLE

In the framework of this tutorial, a practical example was coded in R software and Monolix environment based on the telemetry studies of a kinase inhibitor tested as a therapy for central nervous system-related indications, to provide a pharmacometrician with a template for quick deployment of the workflow and minimize technical procedures required to perform the analysis (Supporting Information S1).

The example is structured into three main folders: "Data," "Models," and "Scripts." Both standardized modeling dataset and data specification files can be found in the "Data" folder. It contains the data from two studies—telemetry and PKs—performed in rats. In a telemetry study with a crossover design, eight male rats received placebo, five and 50 mg of a drug by oral gavage. HR and blood pressure were recorded at 1 h before the dose and for the next 23 h; aggregation interval was 15 min. PK samples were taken 2 h after dose (1 sample per rat). In a PK study, the drug concentration was measured at timepoints 0, 0.5, 1, 2, 4, 8, 12, and 24 h (8 samples per rat) in four rats receiving 5, 10, 25, or 50 mg dose of investigated compound by oral gavage.

The "Models" folder contains the library of the structural models in MLXTRAN format, categorized by the respective stages of the workflow, and final Monolix projects in the "Monolix" subfolder, required for the reproducible example to run out of box.

Similarly, the "Scripts" folder holds one script for the exploratory data analysis and four scripts associated with different elements of the workflow. The scripts are designed to run in R version 4.0.2, with packages tidyverse (version 1.3.1), readxl (version 1.3.1), cowplot (version 1.1.1), PKNCA (version 0.9.5), and RsSimulx (version 1.0.0), and Monolix (version 2020R1). Once sourced, the scripts will store essential results in the newly generated "Results" folder and associated subfolders.

Outputs of the exploratory data analysis (s01_EDA.R) provide priors for the subsequent modeling: nonlinear increase of the drug exposure with dose, notable between-study variability, and apparent physiological threshold in the magnitude of the handling effects, as well as handling effects interfering with the safety effect of the drug.

Script s02_PK.R contains semi-automatic code for calibration and benchmarking of the PK models. The two-compartment PK model with linear excretion from the intestine, saturable absorption described by Michaelis–Menten equation and categorical covariate "Study" on the IA50 parameter was chosen as optimal.

All investigated modeling solutions for handling effects and circadian variations are available in the s03_Placebo.R. As an apparent threshold in the magnitude of the handling effects was identified in placebo data, various handling effect functions were tested according to Table 1. The optimal model contained exponential function for handling effects and between-subject variability on baseline HR parameter.

Implementation of the drug effect, model calibration, and evaluation are available in s04_PKPD.R and is accompanied by an additional graphical analysis of predicted PK and toxicodynamic relationship. Optimal functional relationship was established to be linear without effect compartment and with exponential time adaptation function.

Animal-to-human translation of the drug effect is coded in s05_Translation.R script. The doses of interest for human trial were 10, 50, 100, 140, 200, 300, and 500 mg administered orally once per day. Moderate variability for the volume of distribution and clearance parameters with 30% covariance was implemented for human PK model. The upper limit of 95% prediction interval at steady-state does not exceed 25% threshold for all doses of interest, except the highest (500 mg).

DISCUSSION AND CONCLUSIONS

Mathematical modeling is a powerful and irreplaceable tool for decision making in research and development of new medicines, especially in such delicate matters as FIH dose selection and substantiation.[36,57] Various techniques and data are used to boost confidence in the CV safety of a drug, one being model-based analysis of preclinical telemetry data of hemodynamic markers.[5] While utilizing basic principles of PK/PD modeling in model selection criteria and overall workflow, these analyses are associated with numerous complications, such as small number of animals, prominent handling effects, circadian variations, supratherapeutic doses, sparse PK sampling, etc.[5,19] Altogether, and in no small part due to the narrow timelines assigned for the analyses (2–4 weeks), it substantiates the need for a proper guide covering key steps and specifics of the animal model development, translation, and inference. This tutorial provides a comprehensive sequential workflow, including animal PK model development, modeling of the circadian variations and handling effects, parametrization of the safety effect, and animal-to-human translation, addressing typical challenges in respective sections and supplying the theory with a reproducible example in R and Monolix software.

It should be noted that there are some features of the telemetry study design which would complicate an analysis despite the implemented modeling solutions and assumptions. We recommend discussing the following with the research team beforehand. First, for PK/PD modeling of telemetry data, all animals are assumed to undergo study procedures at the same time, while in reality, drug administration or PK sampling collection of all animals in the study may take up to 30–60 min. Thus, study procedures should be designed to minimize differences in animal handling and the level of animal disturbance as far as possible. In addition, averaging continuous telemetry data in 30-min time intervals or longer impairs the quantification of handling effects and circadian variations. Study duration typically should be designed to accommodate the PK profile and to cover a concentration-response range. However, due to feedback loops and compensatory mechanisms of a CV system, hemodynamic parameters may demonstrate biphasic PD profile. Although the PD changes in the second phase arising from compensatory mechanisms are expected to be considerably lower than the drug-mediated changes in the first phase, study designs can be altered to take the biphasic behavior into consideration. Likewise, the lack of PK sampling at one of drug disposition phases impairs the identification of the optimal structural model. Last, using less than four doses usually significantly complicates the parametrization of the saturable processes.

Modeling solutions, suggested in the current tutorial, cover a wide broad of data complexities that may arise in telemetry studies. However, there are alternative ways to overcome those complexities and build a useful PK/PD model. For instance, instead of separate modeling of circadian variations and handling effect for placebo, one can model placebo-corrected data and skip placebo modeling step of the workflow. Although this can be a working solution, there are some shortcomings with this approach. For example, placebo-corrected data possess higher variance than treatment data without correction and makes it more difficult to identify drug effect. In addition, sometimes the handling effect is diminished or even eliminated in treatment data, and the placebo-correction would lead to subtraction of the handling effect from drug effect and consecutively interferes with estimation of drug effect parameters. Finally, placebo-correction works best within a Latin square framework but is less effective for ascending dose or parallel dose designs because the placebo is only controlling partially for inter-day and inter-group variability. Thus, separate modeling of placebo data and treatment data provide more control for precise estimation of drug-effect parameters and is more generally applicable across study designs.

The implementation of a direct model with a biophase effect compartment rather than indirect response model to capture the delay between PK and toxicodynamic effect is the matter of debate. On the one side, an indirect response model is more appropriate for time lags description not only due to distributional effects but also due to turnover of the biomarker, receptor-transduction, or other rate-limiting pharmacodynamic events.[58] Moreover, the effect compartment model assumes equal time delay for all dose levels and consequently provides poor prediction of maximum response time in case of its dose-dependent nature.[59] On the other side, the choice of whether the drug affects the production or loss of the biomarker is compounded by the lack of evidence about the mechanism of a drug action. Furthermore, human translation of the indirect response model results is challenging as it requires more assumptions to be made and investigations to be done to account differences in the CV system functioning between species. We encourage to use the direct model as a base approach if it provides adequate description of the peak response in addition to studying the dependence of the response peak time on dose and existence of the hypotheses about safety mechanism of action.

Translation of the pharmacological and potential safety effects across species is a challenge in its own way.[60]

Estimation of human PK parameters from preclinical data by PBPK modeling and other approaches is discussed extensively elsewhere and is not in the scope of this paper.[26,50] Moreover, the common rationale for the drug effect adjustment between species in the framework of empirical modeling of telemetry data is limited and is an ongoing subject of active research.[51] Earlier cross-pharma work found that translation from conscious telemetered dog to human was good for corrected QT interval (QTc) but poor for hemodynamic effects, such as HR and diastolic BP.[61] Part of the explanation for the apparent poor translation was posited to be the cross-pharma nature of the dataset with varying study designs and criteria for interpretation. In contrast, the Bhatt et al. paper describes translation experience based on datasets that are much more consistent.[62] In this study, the rat CV model showed good concordance with BP and HR changes in large animal (LA), such as the dog and monkey. Similarly, CV measures of BP and HR in LA showed good concordance to clinical changes. The directionality of BP and HR change was conserved between LA to humans, whereas for rat to LA comparisons, the directionality of change was opposite for 23%–26% of compounds.

In terms of the translational impact, caution should be exercised while translating CV effects directly from rodents to humans in part because of the noted discrepancies and because the level of confidence about the therapeutic exposure is much lower when rodent studies are conducted (early) versus the large animal studies (just prior to FIH studies). Studies in rodents are nevertheless important to identify potential safety signals at early stages of drug development. Data generated in LA prior to FIH are often valuable for translational purposes, both in terms of magnitude and directionality of changes in CV parameters. Overall, a robust translational strategy where possible should be adopted case by case based on the mechanistic knowledge on the pathways driving the observed CV effects.

Although this tutorial presents modeling workflow and decisions criteria for assessment of drug-mediated effect on CV system in the context of safety assessment, general principles of model building remain valid for modeling of drugs effects in any context of concentration-CV effect relationship (e.g., explore PK/PD relationship of drugs designed for treating conditions such as heart failure, hypertension, atrial fibrillation, etc.). However, for drugs with an intended effect on CV system, the translation of results from preclinical disease models to humans can be challenging. A thorough understanding on the similarities/differences in (a) biology between preclinical disease models and humans, (b) the steepness in concentration-response relationship, and (c) a prior understanding on translation is required. While these aspects are important and require proper discussion, they are outside of the scope of the current tutorial.

Finally, this tutorial is also an attempt to formalize decision criteria, based on the reference values of hemodynamic parameters available for humans.[54–56] We use a conservative approach and consider both variability and uncertainty in our predictions; the upper bound of the 95% prediction interval for the peak biomarker value at treatment steady-state is used to compare it with the acceptable ranges. As phase I of the clinical trials is initiated, the incoming ascending dose data should be used continuously to validate the predictions and adjust the model, if necessary, thereby ensuring the safety of the subsequent dosing regimens.

As an alternative, mechanistic models are being developed to capture the intricate relationships between CV parameters and mechanisms of action of a drug.[6,7,43] Extensive validation and application of a quantitative systems model may eventually improve our ability to reliably predict the CV safety between animals and humans. Despite the shortcomings, empirical PK/PD modeling continues to play a vital part in integrated CV safety assessment, allowing for quantitative assessment of CV risk prior to FIH trials, enabling design of dose escalation, and planning of appropriate monitoring and management strategies for CV safety in clinical trials.

In the end, we believe that by following the workflow proposed in this tutorial, a pharmacometrician will be able to conduct a proper and robust nonclinical CV safety analysis and provide quantitative basis for the accurate safety assessment in early clinical trials.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contribution of Amy Pointon, Mike Rolf, Conchi Villar Moas, and Stuart Purbrick for their expert opinion and invaluable advice in conducting the research and in manuscript preparation.

FUNDING INFORMATION

This study was supported by AstraZeneca.

CONFLICT OF INTEREST STATEMENT

V.K., I.V., A.V., K.P., and V.S., are employees of M&S Decisions FZ-LLC, a modeling research consultancy contracted by AstraZeneca. H.K. and R.A. are employees of AstraZeneca and own AstraZeneca stocks or stock options.

ORCID

Victoria Kulesh https://orcid.org/0000-0001-9289-7262
Igor Vasyutin https://orcid.org/0000-0003-0594-7423
Alina Volkova https://orcid.org/0000-0003-2342-2495

Kirill Peskov https://orcid.org/0000-0003-0678-5729
Holly Kimko https://orcid.org/0000-0003-1456-4862
Victor Sokolov https://orcid.org/0000-0001-7066-0811
Ravindra Alluri https://orcid.org/0000-0002-1463-0367

REFERENCES

1. ICH S7A. Safety Pharmacology Studies for Human Pharmaceuticals. Published November 30, 2000. Accessed May 5, 2023. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s-7-safety-pharmacology-studies-human-pharmaceuticals-step-5_en.pdf
2. Onakpoya IJ, Heneghan CJ, Aronson JK. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. *BMC Med*. 2016;14(1):10. doi:10.1186/s12916-016-0553-2
3. Morgan P, Brown DG, Lennard S, et al. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nat Rev Drug Discov*. 2018;17(3):167-181. doi:10.1038/nrd.2017.244
4. Kondic A, Bottino D, Harrold J, et al. Navigating between right, wrong, and relevant: the use of mathematical modeling in preclinical decision making. *Front Pharmacol*. 2022;13:103-110. doi:10.3389/fphar.2022.860881
5. Collins T, Bergenholm L, Abdulla T, et al. Modeling and simulation approaches for cardiovascular function and their role in safety assessment. *CPT Pharmacometrics Syst Pharmacol*. 2015;4(3):175-188. doi:10.1002/psp4.18
6. Snelder N, Ploeger BA, Luttringer O, et al. Drug effects on the CVS in conscious rats: separating cardiac output into heart rate and stroke volume using PKPD modelling. *Br J Pharmacol*. 2014;171(22):5076-5092. doi:10.1111/bph.12824
7. Wallman M, Borghardt JM, Martel E, Pairet N, Markert M, Jirstrand M. An integrative pharmacokinetic-cardiovascular physiology modelling approach based on in vivo dog studies including five reference compounds. *J Pharmacol Toxicol Methods*. 2022;115:107171. doi:10.1016/j.vascn.2022.107171
8. Helmlinger G, Sokolov V, Peskov K, et al. Quantitative systems pharmacology: an exemplar model-building workflow with applications in cardiovascular, metabolic, and oncology drug development. *CPT Pharmacometrics Syst Pharmacol*. 2019;8(6):380-395. doi:10.1002/psp4.12426
9. Collins TA, Rolf MG, Pointon A. Current and future approaches to nonclinical cardiovascular safety assessment. *Drug Discov Today*. 2020;25(7):1129-1134. doi:10.1016/j.drudis.2020.03.011
10. ICH S7B. Non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. Published May 31, 2005. Accessed May 5, 2023. https://www.ema.europa.eu/en/ich-s7b-non-clinical-evaluation-potential-delayed-ventricular-repolarization-qt-interval
11. Mladěnka P, Applová L, Patočka J, et al. Comprehensive review of cardiovascular toxicity of drugs and related agents. *Med Res Rev*. 2018;38(4):1332-1403. doi:10.1002/med.21476
12. Langdon G, Davis JD, McFadyen LM, et al. Translational pharmacokinetic–pharmacodynamic modelling; application to cardiovascular safety data for PF-00821385, a novel HIV agent. *Br J Clin Pharmacol*. 2010;69(4):336-345. doi:10.1111/j.1365-2125.2009.03594.x
13. R Core Team. R: A Language and Environment for Statistical Computing. 2023 Accessed June 21, 2023. https://www.r-project.org/
14. Monolix 2023R1 Documentation. Published 2023. Accessed June 21, 2023. Monolix 2020R1 Documentation.
15. Bussiere JL. Species selection considerations for preclinical toxicology studies for biotherapeutics. *Expert Opin Drug Metab Toxicol*. 2008;4(7):871-877. doi:10.1517/17425255.4.7.871
16. Guth BD. Preclinical cardiovascular risk assessment in modern drug development. *Toxicol Sci*. 2007;97(1):4-20. doi:10.1093/toxsci/kfm026
17. Vargas HM, Amouzadeh HR, Engwall MJ. Nonclinical strategy considerations for safety pharmacology: evaluation of biopharmaceuticals. *Expert Opin Drug Saf*. 2013;12(1):91-102. doi:10.1517/14740338.2013.745851
18. Richens A. Proof of efficacy trials: cross-over versus parallel-group. *Epilepsy Res*. 2001;45(1):43-47. doi:10.1016/S0920-1211(01)00214-5
19. Leishman DJ, Beck TW, Dybdal N, et al. Best practice in the conduct of key nonclinical cardiovascular assessments in drug development: current recommendations from the safety pharmacology society. *J Pharmacol Toxicol Methods*. 2012;65(3):93-101. doi:10.1016/j.vascn.2011.08.006
20. Van Den Buuse M. Circadian rhythms of blood pressure, heart rate, and locomotor activity in spontaneously hypertensive rats as measured with radio-telemetry. *Physiol Behav*. 1994;55(4):783-787. doi:10.1016/0031-9384(94)90060-4
21. Kramer K, van Acker SABE, Voss HP, Grimbergen JA, van der Vijgh WJF, Bast A. Use of telemetry to record electrocardiogram and heart rate in freely moving mice. *J Pharmacol Toxicol Methods*. 1993;30(4):209-215. doi:10.1016/1056-8719(93)90019-B
22. Sivarajah A, Collins S, Sutton MR, et al. Cardiovascular safety assessments in the conscious telemetered dog: utilisation of super-intervals to enhance statistical power. *J Pharmacol Toxicol Methods*. 2010;62(1):12-19. doi:10.1016/j.vascn.2010.05.011
23. Markert M, Trautmann T, Krause F, et al. A new telemetry-based system for assessing cardiovascular function in group-housed large animals. Taking the 3Rs to a new level with the evaluation of remote measurement via cloud data transmission. *J Pharmacol Toxicol Methods*. 2018;93:90-97. doi:10.1016/j.vascn.2018.03.006
24. Morton DB, Hawkins P, Bevan R, et al. Refinements in telemetry procedures: seventh report of BVAAWF/FRAME/RSPCA/UFAW joint working group on refinement, Part A. *Lab Anim*. 2003;37(4):261-299. doi:10.1258/002367703322389861
25. Aylott M, Bate S, Collins S, Jarvis P, Saul J. Review of the statistical analysis of the dog telemetry study. *Pharm Stat*. 2011;10(3):236-249. doi:10.1002/pst.454
26. Jones H, Rowland-Yeo K. Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT Pharmacometrics Syst Pharmacol*. 2013;2(8):e63. doi:10.1038/psp.2013.41
27. Scott Obach R, Baxter JG, Liston TE, et al. The prediction of human pharmacokinetic parameters from preclinical and *in vitro* metabolism data. *J Pharmacol Exp Ther*. 1997;283(1):46.
28. Zhang L, Beal SL, Sheiner LB. Simultaneous vs. sequential analysis for population PK/PD data I: best-case performance. *J Pharmacokinet Pharmacodyn*. 2003;30(6):387-404. doi:10.1023/B:JOPA.0000012998.04442.1f

29. MATLAB: Math. Graphics. Programming Published 2023. Accessed June 21, 2023. https://uk.mathworks.com/products/matlab.html
30. Bauer RJ. NONMEM tutorial part I: description of commands and options, with simple examples of population analysis. *CPT Pharmacometrics Syst Pharmacol*. 2019;8(8):525-537. doi:10.1002/psp4.12404
31. Pillai G, Mentré F, Steimer JL. Non-linear mixed effects modeling—from methodology and software development to driving implementation in drug development science. *J Pharmacokinet Pharmacodyn*. 2005;32(2):161-183. doi:10.1007/s10928-005-0062-y
32. Nguyen THT, Mouksassi MS, Holford N, et al. Model evaluation of continuous data pharmacometric models: metrics and graphics. *CPT Pharmacometrics Syst Pharmacol*. 2017;6(2):87-109. doi:10.1002/psp4.12161
33. Mould D, Upton R. Basic concepts in population modeling, simulation, and model-based drug development—part 2: introduction to pharmacokinetic modeling methods. *CPT Pharmacometrics Syst Pharmacol*. 2013;2(4):38. doi:10.1038/psp.2013.14
34. Pang KS, Rowland M. Hepatic clearance of drugs. I. Theoretical considerations of a "well-stirred" model and a "parallel tube" model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J Pharmacokinet Biopharm*. 1977;5(6):625-653. doi:10.1007/BF01059688
35. Bonate PL. *Pharmacokinetic-Pharmacodynamic Modeling and Simulation*. Springer US; 2011 https://books.google.ru/books?id=6jyVDAEACAAJ
36. EFPIA MID3 Workgroup, Marshall S, Burghaus R, et al. Good practices in model-informed drug discovery and development: practice, application, and documentation. *CPT Pharmacometrics Syst Pharmacol*. 2016;5(3):93-122. doi:10.1002/psp4.12049
37. Marchenko OV, Katenka NV. *Quantitative Methods in Pharmaceutical Research and Development: Concepts and Applications*. Springer International Publishing; 2020 https://books.google.ru/books?id=XTD_DwAAQBAJ
38. Roberts MS, Magnusson BM, Burczynski FJ, Weiss M. Enterohepatic circulation. *Clin Pharmacokinet*. 2002;41(10):751-790. doi:10.2165/00003088-200241100-00005
39. Mould D, Upton R. Basic concepts in population modeling, simulation, and model-based drug development. *CPT Pharmacometrics Syst Pharmacol*. 2012;1(9):6. doi:10.1038/psp.2012.4
40. Chen L, Yang G. Recent advances in circadian rhythms in cardiovascular system. *Front Pharmacol*. 2015;6: 54-60. doi:10.3389/fphar.2015.00071
41. Upton R, Mould D. Basic concepts in population modeling, simulation, and model-based drug development: part 3—introduction to pharmacodynamic modeling methods. *CPT Pharmacometrics Syst Pharmacol*. 2014;3(1):88. doi:10.1038/psp.2013.71
42. Perreau-Lenz S, Pévet P, Buijs RM, Kalsbeek A. The biological clock: the bodyguard of temporal homeostasis. *Chronobiol Int*. 2004;21(1):1-25. doi:10.1081/CBI-120027984
43. Fu Y, Taghvafard H, Said MM, et al. A novel cardiovascular systems model to quantify drugs effects on the inter-relationship between contractility and other hemodynamic variables. *CPT Pharmacometrics Syst Pharmacol*. 2022;11(5):640-652. doi:10.1002/psp4.12774
44. Visser SAG, Sällström B, Forsberg T, Peletier LA, Gabrielsson J. Modeling drug- and system-related changes in body temperature: application to clomethiazole-induced hypothermia, long-lasting tolerance development, and circadian rhythm in rats. *J Pharmacol Exp Ther*. 2006;317(1):209-219. doi:10.1124/jpet.105.095224
45. Mohrman DE, Heller LJ. *Cardiovascular Physiology 8/E*. McGraw-Hill Education; 2014 https://books.google.ru/books?id=hbEJAgAAQBAJ
46. Louizos C, Yáñez JA, Forrest ML, Davies NM. Understanding the hysteresis loop conundrum in pharmacokinetic/pharmacodynamic relationships. *J Pharm Pharm Sci*. 2014;17(1):34-91. doi:10.18433/J3GP53
47. Csajka C, Verotta D. Pharmacokinetic–pharmacodynamic modelling: history and perspectives. *J Pharmacokinet Pharmacodyn*. 2006;33(3):227-279. doi:10.1007/s10928-005-9002-0
48. Wang J, Li W. Test hysteresis in pharmacokinetic/pharmacodynamic relationship with mixed-effect models: an instrumental model approach. *J Biopharm Stat*. 2014;24(2):326-343. doi:10.1080/10543406.2013.859149
49. De Buck SS, Sinha VK, Fenu LA, Nijsen MJ, Mackie CE, Gilissen RAHJ. Prediction of human pharmacokinetics using physiologically based modeling: a retrospective analysis of 26 clinically tested drugs. *Drug Metab Dispos*. 2007;35(10):1766-1780. doi:10.1124/dmd.107.015644
50. Zhao P, Rowland M, Huang SM. Best practice in the use of physiologically based pharmacokinetic modeling and simulation to address clinical pharmacology regulatory questions. *Clin Pharmacol Therapeut*. 2012;92(1):17-20. doi:10.1038/clpt.2012.68
51. Parkinson J, Visser SAG, Jarvis P, et al. Translational pharmacokinetic–pharmacodynamic modeling of QTc effects in dog and human. *J Pharmacol Toxicol Methods*. 2013;68(3):357-366. doi:10.1016/j.vascn.2013.03.007
52. Al-Sallami HS, Cheah SL, Han SY, et al. Between-subject variability: should high be the new normal? *Eur J Clin Pharmacol*. 2014;70(11):1403-1404. doi:10.1007/s00228-014-1740-8
53. Kümmel A, Bonate PL, Dingemanse J, Krause A. Confidence and prediction intervals for Pharmacometric models. *CPT Pharmacometrics Syst Pharmacol*. 2018;7(6):360-373. doi:10.1002/psp4.12286
54. National Institutes of Health. Pulse. Accessed May 16, 2023. https://medlineplus.gov/ency/article/003399.htm
55. Unger T, Borghi C, Charchar F, et al. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension*. 2020;75(6):1334-1357. doi:10.1161/HYPERTENSIONAHA.120.15026
56. Left ventricular function. Accessed May 5, 2023. https://123sonography.com/ebook/left-ventricular-function
57. FDA. Population Pharmacokinetics. Guidance for Industry. Accessed May 16, 2023. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/population-pharmacokinetics
58. Jusko WJ, Ko HC. Physiologic indirect response models characterize diverse types of pharmacodynamic effects. *Clin Pharmacol Therapeut*. 1994;56(4):406-419. doi:10.1038/clpt.1994.155

59. Wakelkamp M, Alván G, Paintaud G. The time of maximum effect for model selection in pharmacokinetic–pharmacodynamic analysis applied to frusemide. *Br J Clin Pharmacol*. 1998;45(1):63-70. doi:10.1046/j.1365-2125.1998.00637.x
60. Leenaars CHC, Kouwenaar C, Stafleu FR, et al. Animal to human translation: a systematic scoping review of reported concordance rates. *J Transl Med*. 2019;17(1):223. doi:10.1186/s12967-019-1976-2
61. Ewart L, Aylott M, Deurinck M, et al. The concordance between nonclinical and phase I clinical cardiovascular assessment from a cross-company data sharing initiative. *Toxicol Sci*. 2014;142(2):427-435. doi:10.1093/toxsci/kfu198
62. Bhatt S, Northcott C, Wisialowski T, Li D, Steidl-Nichols J. Preclinical to clinical translation of hemodynamic effects in cardiovascular safety pharmacology studies. *Toxicol Sci*. 2019;169(1):272-279. doi:10.1093/toxsci/kfz035

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kulesh V, Vasyutin I, Volkova A, et al. A tutorial for model-based evaluation and translation of cardiovascular safety in preclinical trials. *CPT Pharmacometrics Syst Pharmacol*. 2024;13:5-22. doi:10.1002/psp4.13082